

IN THE CLAIMS

Please amend the claims as follows:

Claims 1-80 (Cancelled).

81. (Currently Amended) A method of simultaneously detecting two three or more antigens in a sample, comprising simultaneously contacting a sample in an automated staining device, which has been previously simultaneously contacted with a primary antibody cocktail comprising at least one first primary antibody, [[and]] at least one second primary antibody, and at least one third primary antibody in a buffered aqueous solution at a pH from about 5.7 to about 7.3 suitable to stabilize the primary antibody cocktail, with a composition comprising at least one first secondary antibody, [[and]] at least one second secondary antibody, and at least one third secondary antibody wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety, and wherein the composition comprises a buffer suitable to stabilize the first and second secondary antibodies; and simultaneously detecting the formation of at least two three antigen-antibody complexes on the sample.

82-84. (Cancelled)

85. (Currently Amended) The method of Claim 81, which comprises detecting at least four antigens in a sample and wherein the method comprises contacting the sample, after detecting the formation of the antigen-antibody complexes, with wherein the primary antibody cocktail further comprises at least a third and a fourth primary antibody; and the method further comprises simultaneously detecting the formation of at least third and a fourth antibody-antigen complex complexes on the sample.

86. (Cancelled)

87. (Currently Amended) A method of simultaneously detecting ~~two~~ three or more antigens in a sample, comprising simultaneously contacting a sample on an automated staining device with a primary antibody cocktail comprising at least one first primary antibody, [[and]] at least one second primary antibody, and at least one third primary antibody in a buffered aqueous solution at a pH from about 5.7 to about 7.3 suitable to stabilize the primary antibody cocktail, and subsequently simultaneously contacting the sample with a composition comprising at least one first secondary antibody, [and] at least one second secondary antibody, and at least one third secondary antibody, wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety, and wherein the composition comprises a buffer suitable to stabilize the first and second secondary antibodies; and

simultaneously detecting the formation of at least ~~two~~ three antigen-antibody complexes on the sample.

Claims 88-90 (Cancelled).

91. (Currently Amended) The method of Claim 87, which comprises detecting at least four antigens in a sample and wherein the ~~method comprises contacting the sample, after detecting the formation of the antigen-antibody complexes, with primary antibody cocktail further comprises~~ at least ~~a~~ third and a fourth primary antibody; and the method further comprises detecting the formation of at least ~~third and a~~ fourth antibody-antigen complexes complex on the sample.

92. (Cancelled).

Claims 93-103 (Cancelled).

104. (New) The method of Claim 81, wherein the primary antibody cocktail comprises one or more of the following pairs of antibodies:

CD31 and Ki-67;

CD34 and Factor XIII subunit a;

CDX2 and CK7;

Ki-67 and Caspase-3;

M30 and Ki-67;

LCA and S100;

Kappa light chain + lambda light chain.

p63 and P504S;

CK5/6 and Calretinin

Ki-67 and ER; or ER + K-67;

p16 and Ki-67

CD3 and Ki-67;

PAX-5 and CD5;

CD4 and CD8;

CD10 and Cyclin D1

CD23 and CD5

PR and ER

GFAP and microglia

Ki-67 and microglia

Neurofilament and microglia

CD56 and Synaptophysin

GCDFP-15 and Mammaglobin

105. (New) The method of Claim 87, wherein the primary antibody cocktail comprises one or more of the following pairs of antibodies:

CD31 and Ki-67;
CD34 and Factor XIII subunit a;
CDX2 and CK7;
Ki-67 and Caspase-3;
M30 and Ki-67;
LCA and S100;
Kappa light chain and lambda light chain.
p63 and P504S;
CK5/6 and Calretinin
Ki-67 and ER; or ER + K-67
p16 and Ki-67
CD3 and Ki-67;
PAX-5 and CD5;
CD4 and CD8;
CD10 and Cyclin D1
CD23 and CD5
PR and ER
GFAP and microglia
Ki-67 and microglia
Neurofilament and microglia
CD56 and Synaptophysin
GCDFP-15 and Mammaglobin

106. (New) The method of Claim 81, wherein the primary antibody cocktail comprises one or more of the following groups of antibodies

CK5 and CK14 and p63 and P504S

CK5 and p63 and HMW CK and P504S

CK5 and p63 and 34betaE12 and P504S

Tyrosinase and MART-1 and MART-1 and S100

MART-1 and Tyrosinase and CDX-2 and PSA and TTF1 and Synaptophysin (6)

CD5 and p63 and CK8/18 and/or CK8 and/or CK18

GFAP and microglia and Ki-67

107. (New) The method of Claim 85, which comprises detecting at least five or six antigens in a sample and wherein the primary antibody cocktails further comprises at least a 5 or 6 primary antibodies; and wherein the method further comprises detecting the formation of at least a fifth or sixth antibody-antigen complexes on the sample.

108. (New) The method of Claim 91, which comprises detecting at least five or six antigens in a sample and wherein the primary antibody cocktails further comprises at least a 5 or 6 primary antibodies; and wherein the method further comprises detecting the formation of at least a fifth or sixth antibody-antigen complexes on the sample.

109. (New) The method of Claim 81, wherein the buffered aqueous solution comprises is an aqueous solution comprising Tris-HCl, sodium azide, BSA, and hydrochloric acid.

110. (New) The method of Claim 87, wherein the buffered aqueous solution comprises is an aqueous solution comprising Tris-HCl, sodium azide, BSA, and hydrochloric acid.

111. (New) The method of Claim 81, wherein prior to simultaneously contacting with the primary antibody cocktail, the method further comprises:

placing a heat sensitive pH indicating retrieval solution in a pressure cooker with a temperature control, a timer control, a temperature display, and a pressure gauge, wherein

said cooker is operable within a predetermined temperature range and a predetermined pressure range, and is arranged to display an actual temperature, wherein said pH indicating retrieval solution is arranged for changing color within said temperature range to indicate a pH change to a value within predetermined range of pH; and

placing said biological sample in said pH indicating retrieval solution; setting a heating temperature with said temperature control; setting a timer period with said timer control; activating said cooker; recording an actual temperature shown on said temperature display and an actual pressure shown on said pressure gauge after said heating temperature is reached; opening said cooker after heating; checking said pH indicating retrieval solution for color change which indicates that said range of pH has been reached; and recording pH indicated by said pH indicating retrieval solution.

112. (New) The method of Claim 81, wherein prior to simultaneously contacting with the primary antibody cocktail, the method further comprises:

placing a heat sensitive pH indicating retrieval solution in a pressure cooker with a temperature control, a timer control, a temperature display, and a pressure gauge, wherein said cooker is operable within a predetermined temperature range and a predetermined pressure range, and is arranged to display an actual temperature, wherein said pH indicating retrieval solution is arranged for changing color within said temperature range to indicate a pH change to a value within predetermined range of pH; placing said biological sample in said pH indicating retrieval solution; placing a heat and pressure sensitive steam strip in said cooker, wherein said steam strip is arranged to change color within said temperature range and said pressure range for indicating temperature and pressure changes; setting a heating temperature with said temperature control; setting a timer period with said timer control; activating said cooker; recording an actual temperature shown on said temperature display

and an actual pressure shown on said pressure gauge after said heating temperature is reached; opening said cooker after heating; checking said pH indicating retrieval solution for color change which indicates that said range of pH has been reached; recording pH indicated by said pH indicating retrieval solution; and checking said steam strip for color change which indicates that said temperature range and said pressure range have been reached, so as to back up reading of said temperature display and said pressure gauge for reliability and accuracy.

113. (New) The method of Claim 81, wherein prior to simultaneously contacting with the primary antibody cocktail, the method further comprises:

placing a retrieval solution in a pressure cooker with a temperature control, a timer control, a temperature display, and a pressure gauge, wherein said cooker is operable within a predetermined temperature range and a predetermined pressure range, and is arranged to display an actual temperature; placing said biological sample in said retrieval solution; setting a heating temperature with said temperature control; setting a timer period with said timer control; activating said cooker; recording an actual temperature shown on said temperature display and an actual pressure shown on said pressure gauge after said heating temperature is reached; opening said cooker after heating; placing a pH strip in said retrieval solution; comparing said pH strip to a heat adjusted color chart arranged for accurately reading said pH strip after said pH strip is heated by said retrieval solution; and recording pH indicated by said pH strip.

114. (New) The method of Claim 81, wherein prior to simultaneously contacting with the primary antibody cocktail, the method further comprises:

placing a retrieval solution in a pressure cooker with a temperature control, a timer control, a temperature display, and a pressure gauge, wherein said cooker is operable within a predetermined temperature range and a predetermined pressure range, and is arranged to display an actual temperature; placing said biological sample in said retrieval solution;

placing a heat and pressure sensitive steam strip in said cooker, wherein said steam strip is arranged to change color within said temperature range and said pressure range for indicating temperature and pressure changes; setting a heating temperature with said temperature control; setting a timer period with said timer control; activating said cooker; recording an actual temperature shown on said temperature display and an actual pressure shown on said pressure gauge after said heating temperature is reached; opening said cooker after heating; placing a pH strip in said retrieval solution; comparing said pH strip to a heat adjusted color chart arranged for accurately reading said pH strip after said pH strip is heated by said retrieval solution; recording pH indicated by said pH strip; and checking said steam strip for color change which indicates that said temperature range and said pressure range have been reached, so as to back up reading of said temperature display and said pressure gauge for reliability and accuracy.

115. (New) A method of detecting two or more antigens in a biological sample, comprising

placing a heat sensitive pH indicating retrieval solution in a pressure cooker with a temperature control, a timer control, a temperature display, and a pressure gauge, wherein said cooker is operable within a predetermined temperature range and a predetermined pressure range, and is arranged to display an actual temperature, wherein said pH indicating retrieval solution is arranged for changing color within said temperature range to indicate a pH change to a value within predetermined range of pH;

placing said biological sample in said pH indicating retrieval solution; setting a heating temperature with said temperature control; setting a timer period with said timer control; activating said cooker; recording an actual temperature shown on said temperature display and an actual pressure shown on said pressure gauge after said heating temperature is

reached; opening said cooker after heating; checking said pH indicating retrieval solution for color change which indicates that said range of pH has been reached; and recording pH indicated by said pH indicating retrieval solution,

simultaneously contacting the biological sample, which has been previously simultaneously contacted with a primary antibody cocktail comprising at least one first primary antibody and at least one second primary antibody, with a composition comprising at least one first secondary antibody and at least one second secondary antibody, wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety, and wherein the composition comprises a buffer suitable to stabilize the first and second secondary antibodies; and

detecting the formation of at least two antigen-antibody complexes on the sample.